MECHANISM OF OVERGROWTH IN PLANTS.

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I. Introductory.

For 12 years I have been an eager student of overgrowths in plants, partly on account of agricultural phases of the problem which are of economic importance but chiefly because they have seemed to me to offer a clue which might lead to the solution of the greater and very obscure problem of the origin of malignant human and animal tumors.

For a long time I have believed that the direct cause of these plant tumors (of all malignant tumors for that matter) must be chemical substances liberated in the tissues by parasites. It is not a far cry to such a view, especially where parasites are known to cause the overgrowth, and no doubt many other persons have held the same view and have stated it more or less definitely. I expressed it clearly in 1911 in our first crown gall bulletin (U. S. Dept. of Agric., B. P. I., Bul. 213, p. 175).

The difficulty has been to determine the nature of these chemical substances. This is still unsolved so far as relates to the products of gall-forming larvæ of all kinds, and apparently must so remain until they can be grown in quantity in pure culture so as to give to the chemist an abundance of material for his studies. The chemist is very greedy of material and without a great abundance he can seldom accomplish much. Various gall-forming fungi and bacteria offer easier problems because they can be cultivated in flasks on simple culture media in any desired quantity and their products determined with a minimum of labor.

This, rather than the analysis of tumors, is, I am satisfied, the proper method of procedure, because the cells of a tumor are only the cells of a plant or animal grown under an abnormal stimulus,

which stimulus, it is very likely, is not only very minute in quantity but also used up during the growth of the tumor cells, that is, converted into something quite different and entirely inoffensive. For this reason analyses of tumor tissue should give only about the same kind and quantity of products as normal tissues in which there is an equally rapid movement of food-stuffs, and in which there is an equally rapid growth, and this is about what tumor analyses thus far have shown. In flask cultures, on the contrary, the products of parasitic growth accumulate and can be locked up for future study.

What I have done, in addition to speculating, is to grow various strains of *Bacterium tumefaciens,* the crown-gall organism, in pure culture in quantity in cotton-plugged Jena glass flasks for chemical examination. Being a member of the United States Department of Agriculture, the greatest coöperative research institution in the world, it has been easy to come into touch with expert organic chemists and through them to have determined for me the various substances produced by the crown-gall organism out of river water, peptone and grape sugar, *i. e.,* substances corresponding to or approximating those which occur naturally in the cells of the plant. These flasks were inoculated with great care and watched as to their behavior. Before turning them over to the chemist, Petri-dish agar plates were poured from each one to determine whether they were still pure cultures. The analyses were then made *pari passu* with inoculations into susceptible plants to determine whether the cultures were still pathogenic. In this way various flasks were tested and worked up separately, with, in the main, concordant results. The inoculated flasks behaved properly, the agar-poured plates yielded uniform normal-looking colonies, and subcultures from colonies derived from each flask were subsequently inoculated into plants with the production of crown galls in every case except that of the isolation from poplar, which was known to be no longer pathogenic when the experiment was begun. All of the flasks had remained pure cultures and were in good condition for the chemist, who worked them over quickly. These cultures originated from single colonies selected from agar-poured plates made from tumors on hop, Paris daisy, rose and poplar, and represent at least two strains of the crown-gall organism.

II. Chemical Findings.

Slide No. 1 (Table I.) shows the chemical findings. On this slide I have starred the substances with which I have now produced overgrowths in plants and have italicized those which Dr. Jacques had previously found in his experiments on animal eggs to be most effective in causing unfertilized eggs to begin to grow.[1] That there should be so many of these egg-starting substances excreted by a tumor-producing parasite is not only astonishing but extremely suggestive. All of them are substances which pass readily through protoplasmic membranes.

TABLE I.

Showing Products of *Bacterium tumefaciens.*

* *Ammonia*	Acetone
* *Amines*	* *Acetic Acid*
* Aldehyd	* *Formic Acid*
Alcohol	*Carbonic Acid* (?)

I have added carbonic acid of my own accord, since I did not ask the chemists to search for it: (1) because the crown-gall schizomycete must be very unlike other organisms if it does not produce some carbonic acid as the result of its growth, although certainly not enough is developed to appear in fermentation tubes as the gas CO_2; (2) because the excess of leaf-green (chlorophyll bodies, which assimilate CO_2) in the deeper tissue of galls on Paris daisy suggests presence of carbonic acid in excess of these tissues; and (3) because carbonic acid also is one of those substances found by Loeb to stimulate the development of unfertilized eggs. My experiments are still under way, none of them are really completed, and today I will only call your attention to a few of my results, some of which have already been published,[2] while others are here mentioned for the first time. I would call attention especially to the substances the names of which I have starred as compounds with which to experiment singly and combined, and in a great variety of dilutions. With each one of these substances, in the absence of bacteria, I have obtained on suitable plants decided overgrowths,

[1] Loeb, "Artificial Parthenogenesis and Fertilization," 1913.

[2] *Jour. Agric. Research,* January 29, 1917.

growths which I think I am warranted in designating as incipient crown galls. The overgrowths I have obtained are small, as was to be expected from the application of a single slight stimulus. They do not continue to grow because they are the response to an abnormal outside influence of very limited duration, or to put it in another way, because there is no parasitic organism back of the growth, as in the case of the natural crown gall, to continually stimulate it by means of its excretions. In this particular, that is in the continuous slow introduction of these substances into the tissues after the manner of the parasite, I have not yet found it possible to imitate nature, but in view of the overgrowths I have obtained by a single slight stimulus it can no longer be doubted that even in the absence of the bacteria the slow continual oozing into growing tissues of the dilute acids, alkalies and other substances named would produce a crown gall of any size desired. So long as the stimulus is applied, and in nature it will be applied as long as the bacteria are present in the tissues and continue to grow, so long the growing tissues must respond.

Before passing I wish once more to call attention to the italicized names, and to urge all students of overgrowths to read Dr. Loeb's book, since these tumor-producing substances, as I have said, are those Dr. Loeb has found most active in starting the development of animals out of unfertilized eggs.

We will now pass to slides showing results obtained with ammonia, dimethylamine, formaldehyde, acetic acid, and formic acid (slides exhibited).

III. The Mechanism of Overgrowths.

We now come to the inquiry embodied in the title of this paper —what is the mechanism of these overgrowths? Is it a chemical or physical action? It is plain that the response is due to soluble substances poured out, as a result of their metabolism, by parasites present in the tissues, but given off in such small quantities that they act not as a poison but as a growth-stimulus. That many poisons when applied in minute doses do act as stimulants of one kind or another is already well known, both in medicine and in agriculture. That suspension colloids would be precipitated, pro-

teins split, and very marked osmotic disturbances set up within the mechanism of the delicately balanced colloids of the cell upon introduction of these dilute, non-plasmolyzing bacterial acids, alkalis and other products, must be apparent to anyone who is at all familiar with the colloidal chemistry of the cell; and later, by means of physical chemistry, we ought to be able to determine at least some of the physical-chemical steps in the process of the abnormal cell division brought about by these disturbing substances.

For the present I interpret the growth in crown gall as due primarily to a physical cause, viz., to an increase in the osmotic pressure due to the heaping up locally of various soluble substances excreted by the bacteria as a result of their metabolism. This would lead to a movement of equalization. Water containing dissolved food stuffs would move toward the tumor and the stimulating acids and alkalies would move outward so that theoretically the strongest tendency to overgrowth should occur in the periphery of the tumor where, as a matter of fact, it does occur. Also in malignant human tumors the growth is peripheral. *Why is it peripheral?* If this hypothesis is correct we ought to be able to detect at least a slight difference between the concentration of salts in fluids on the periphery of a tumor and in the normal tissues just beyond it. This, I believe, could be determined best electrically, although, if the difference is considerable, the coarser method of extraction of the juice of tumors and of adjacent sound tissues and determination whether there is any depression of the freezing point in the former might yield interesting results. One test made for me by Mr. Rodney B. Harvey indicated that there is a concentration of substances in the juice of daisy tumors, *i. e.*, there was a lowering of the freezing point, but no thorough study has been made. This I contemplate taking up in conjunction with physicists of the Department of Agriculture.

The reason I have for thinking the phenomena of plant overgrowth is primarily physical is the fact that it can be obtained by a great variety of substances not the products of parasites, anything in fact, which disturbs tissue equilibriums without destroying cells, seems to be capable of causing overgrowths, which cease, of course,

as soon as the stimulus is exhausted. (See Mechanism of Tumor Growth in Crown Gall, *in* Jour. Agric. Research, Jan. 29, 1917.)

I have been asked in what way these overgrowths differ from the ordinary healing of wounds. The growth while excessive is probably not fundamentally different from a wound reaction, but then, for that matter, we may regard all tumors as so many efforts at healing which come to naught because they are continually modified and frustrated by the presence of a parasite, or in animal cancers, let us say, since we do not know their cause, by an abnormal and oft repeated stimulus of some sort, most easily explained in the absence of exact data by the hypothesis of a parasite, especially since the same phenomenon in plants can now be referred to a definite microörganism.

IV. The Kind of Tumor Depends on the Type of Cells Stimulated.

The first crown galls I studied seemed to me to be overgrowths of the conjunctive tissues and most of our many inoculations up to the end of 1915 produced that type of tumor which corresponds, I believe, to overgrowths of the connective tissue of animals and which I have called plant sarcomas.

We had found indeed, as early as 1908–9, and had produced by bacterial inoculation, plant tumors bearing roots, but the full meaning of this discovery, as related to cancer, did not occur to me until early in 1916, when I found crown-gall tumors bearing leafy shoots on some of our inoculated hothouse geraniums. Beginning with this discovery I made numerous inoculations in the leaf axils of various plants which resulted in the production of leafy tumors, and subsequently I produced them freely on leaves and on cut internodes where no buds occur normally. Tumors bearing roots have also been produced by us on the top of plants, and in one cut internode of tobacco I succeeded in producing a tumor which bore flower buds. These perishable root-bearing and shoot-bearing tumors I regard as plant embryomas and have so described them.[3]

These experiments render it probable that every growing organ

[3] *Journ. Cancer Research,* April, 1916, p. 241.

normally contains multipotent or totipotent cells which usually remain dormant, but which under a strong stimulus are capable of developing into either the whole organism or into some considerable part of it, what is developed out of them depending on the degree of differentiation of the cells at the time they are stimulated. We may regard these leafy shoots (produced sometimes in great numbers where no buds occur normally) either as going to show that potentially there is no difference between germ-cells and young somatic cells, or else that dormant "germ-cells" are widely and abundantly distributed among the somatic cells, ready to develop into the whole or a considerable part of the organism whenever a sufficient stimulus is applied. Those who wish further details respecting these recently produced and peculiar crown galls containing fragments of the embryo plant are referred to a special paper on the subject in the "Bulletin of the Johns Hopkins Hospital" for September, 1917.

V. Bearings of These Discoveries.

That these discoveries have many interesting bearings goes without argument. Some of these bearings may be mentioned:

(*a*) On the origin of insect, nematode and fungous galls;

(*b*) On the formation of thyloses in vessels;

(*c*) On the origin, through absorbed poisons, of certain plant diseases whose etiology is very obscure, such as peach yellows, peach rosette, and the various mosaic diseases;

(*d*) On the origin, in the same way, of various plant and animal monstrosities;

(*e*) On various problems of modification by slight changes in environment;

(*f*) On possibility of normal wide distribution of dormant germ-cells among somatic cells;

(*g*) And, finally, on the etiology of various human and animal tumors.

VI. Earlier Work and Reasons Why it Remained Sterile.

I must here refer to some earlier work which remained sterile so far as any influence on tumor etiology is concerned (*a*) because

done under the idea that tumors are due to the existence of specific overgrowth stimuli; (*b*) because done with substances which could by no possibility be conceived to be the product of parasites; and still more (*c*) because the experiments fell on stony ground, that is into the unreceptive minds of a generation of pathologists preoccupied with quite other ideas and generalizations respecting tumor growth.

I refer more particularly to Dr. Hermann von Schrenk's papers (1903 and 1905) on intumescences in cauliflower plants due to copper salts,[4] and to Dr. Bernhard Fischer's paper on overgrowths of epithelium due to the injection of scarlet red and indophenol into rabbit's ears.[5]

Fischer's paper in particular pointed the way clearly toward the solution of the cancer problem, but it was received very coldly and he became discouraged, and no one else took up the suggested clue.

What Fischer obtained was downgrowths of epithelium into the connective tissue, strikingly suggestive of epithelioma, but, because these invading epithelial cells subsequently ceased to grow, with disappearance of the stimulus, and were finally absorbed, as one might reasonably have predicted would be the case, they were held to throw no light on the cancer problem; but if specialists had then assumed that quite other substances than scarlet red and indophenol can cause overgrowths, as we now know, and that some of the substances may be the products of the tumor-producing parasites, as also we now know, how suddenly luminous the whole subject would have become and what an incentive it would have given, and *still gives,* to further research!

[4] See especially Report of Missouri Botanical Garden, 1905, p. 125.

[5] Muenchner med. Wochenschrift, 1906, p. 2041.